

**HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2:
(E. COLI, B. MEGATERIUM, RECOMBINANT ANTIGEN)
HIVAB™ HIV-1/HIV-2 (rDNA) EIA**

NOTE CHANGES HIGHLIGHTED

EUSA

NAME AND INTENDED USE

HIVAB™ HIV-1/HIV-2 (rDNA) EIA IS AN *IN VITRO* ENZYME IMMUNOASSAY FOR THE QUALITATIVE DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUSES TYPE 1 AND/OR TYPE 2 (HIV-1/HIV-2) IN HUMAN SERUM OR PLASMA.

68-0158/R12

WARNING: A SOFTWARE UPGRADE AND/OR PROTOCOL EDITS MAY BE REQUIRED PRIOR TO IMPLEMENTING THIS ASSAY. PLEASE CONTACT YOUR LOCAL CUSTOMER SUPPORT CENTER.



ABBOTT LABORATORIES
Diagnostics Division

CUSTOMER SUPPORT CENTER USA
1-800-521-9100

© Abbott Laboratories, 1996
Abbott Laboratories
Diagnostics Division
Abbott Park, IL 60064
U.S. License No. 43
Lot No. 9A77

December 1996

Printed in U.S.A.

Some components of the prepack powder Substrate Acid. For a specific listing, refer to the **REAGENTS** section of the package insert. The components containing Substrate Acid are classified per applicable European Economic Community (EEC) Directives as Hazardous (H). The following are the appropriate Risk (R) and Safety (S) phrases:

- R02** Harmful if swallowed.
R302 Harmful if swallowed. May irritate your mouth and skin.
R312 Harmful if absorbed. May irritate your eyes.
R313 Keep away from food, drink and animal feedstuffs.
R360 Wear suitable protective clothing.
S06 If swallowed, seek medical advice immediately and show the container or label.

The OPD tablet listed in the **REAGENTS** section of this package insert contain a Phenylenediamine + 2 HCl salt Substrate Compound. The OPD tablets are classified per applicable European Economic Community (EEC) Directives as Hazardous (H). The following are the appropriate Risk (R) and Safety (S) phrases:

- R020** Harmful if swallowed. May irritate your mouth and skin.
R302 Harmful if swallowed. May irritate your mouth and skin.
R312 Harmful if absorbed. May irritate your eyes.
R313 Keep away from food, drink and animal feedstuffs.
S06/330 Wear suitable protective clothing, gloves and eye/face protection.
S08 If swallowed, seek medical advice immediately and show the container or label.

Handling Precautions

- Do not use until beyond the expiration date.
 - Do not use reagents from different lots.
- NOTE:** Any OPD reagent lot or 1 N Substrate Acid lot may be used with any HEMOTEC EIA kit.
- Avoid excessive contamination of reagents when removing aliquots from the reagent vials. Use of disposable pipette tips is recommended.
 - Do not measure OPD reagents to strong light during storage or incubation.
 - Avoid contact of the OPD Substrate Solution or 1 N Substrate Acid with any reading agent. Do not allow OPD Substrate Solution to come in contact with any metal parts. Prior to use, immerse glassware to be used for OPD Substrate Solution thoroughly with 1 N Acid solution or hydrochloric acid approximately 10% into a container containing sufficient fresh volume of distilled water if the reagent is not already washed. Rinse the glassware in bottle and/or gently tap bottle for drainage. Do not allow fluids with detergent attached to remain.
 - Use a clean, dedicated dispenser for the diluted conjugate to avoid contamination.
 - The Negative and HCV 1 Positive Controls, and the HCV 2 Positive Control, as provided, should be handled the same way as specimens.

INSTRUCTION FOR PREPARATION OF DILUTED CONJUGATE

- Conjugate Concentrate and Conjugate Diluent should be brought to room temperature before using.
- Carefully empty the contents of a Conjugate Concentrate vial into a vial of Conjugate Diluent. This can be done more efficiently by slowly squeezing the small vial so that 2-3 mm of conjugate remains at the bottom after the opening of the large vial is fully submerged. Rinse the small vial with water provided in the Conjugate Diluent vial.
- Reverse the large vial. Mix thoroughly by slowly inverting the vial several times. Do not vortex.
- Allow diluted conjugate to equilibrate at room temperature for approximately 30 minutes before use.
- Once a diluted conjugate is sufficient for all tests (100 Test Kits/400 tests/1,000 tests and tubes), Conjugate is stable for 21 days, though full sensitivity is maintained after dilution when stored at 2-8°C. Bring to room temperature before using.

INSTRUCTIONS FOR PREPARATION OF OPD SUBSTRATE SOLUTION

Bring OPD Reagents to room temperature (20 to 30°C).

CAUTION: Do not open OPD Tablet bottle until it is at room temperature.

At least 6 minutes, but not more than 30 minutes prior to Color Development, remove the OPD Substrate Solution by decanting the OPD Color Development + 2 HCl reagent or Diluent for OPD. **DO NOT USE A VIAL SET THAT IS NOT INTACT.**

Bring clean pipettes and reaction components such as plastic ware to add washed and distilled water (meq) previously from the product lot.

- Transfer into a suitable container 5 mL of Diluent for OPD for each bottle to be assayed.
- Transfer appropriate amount of OPD Tablets into OPD Preparation Chart, and measured amount of Diluent for OPD using a volumetric flask or graduated cylinder, sufficient to bring to volume. A 100 mL or 200 mL water and/or distilled water. Allow to stand for 10 minutes. Do not vortex. The OPD Substrate Solution (white when the tablets are dissolving). The OPD SUBSTRATE SOLUTION (see the label) will settle into a precipitate. (For PREPARATION AND USE, see the OPD kit's instruction.)
- Continue to decanting by Color Development, until certain degree is when a homogeneous solution, remove all bubbles from top, and pipette transfer into vial to use.

OPD PREPARATION CHART

No. of Tests	Tablets	Diluent
12	1	3 mL
20	2	10 mL
30	3	15 mL
50	4	20 mL
75	5	30 mL
100	6	40 mL
120	7	50 mL
150	8	60 mL
180	10	80 mL

NOTE: OPD Substrate Solution is supplied for each specimen or Control as well as for each substrate blank. Laboratories using the COMBINATION HEMOTEC Control are instructed to use the OPD reagent and OPD Substrate Solution for subsequent printing.

STORAGE INSTRUCTIONS

- Store kit reagents at 2 to 8°C. OPD Tablets and 1 N Substrate Acid may be stored at 2 to 30°C.
- Bring all reagents to room temperature (20 to 30°C) for use and return them to original containers immediately after use.
- CAUTION:** Do not open OPD Tablet bottle until it is at room temperature.
- Return decanted bags to OPD Tablet bottle at all times during storage.
- The OPD Substrate Solution **MUST** be stored at room temperature and **MUST** be used within 30 minutes. Do not attempt to dilute/adjust.
- Reagent containers to avoid light, and cap bottles to storage.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

The OPD Substrate Solution (plus Diluent for OPD) should be carefully inspected before use. A color change later indicates that the reagent has been contaminated and may be discarded.

An absorbance value above 0.300 for anti-HCV 1 Positive Control replicate within an absorbance value of less than 0.050 for other HCV 1 Positive Control replicate may indicate technique errors or deterioration of the kit reagents or OPD reagents. Such reagents should be replaced.

SPECIMEN COLLECTION AND PREPARATION

HEMOTEC HCV-1/2 (VNA) EIA may be performed on either frozen serum or plasma.

- If specimens are to be stored, they may be stored at 2 to 8°C for a maximum of 18 days. For long term storage, the specimens should be stored frozen. Samples that are frozen after 3 months may show no performance difference but.
- If specimens are to be analyzed, they should be stored and handled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.
- The HEMOTEC HCV-1/2 (VNA) EIA can be used with serum containing sodium citrate (3.8% sodium citrate) or plasma containing heparin, EDTA, citrate and CPDA-1.
- Clean, well-washed specimens should be used. Separate blood from plasma from the clot or use only as much as possible to avoid hemolysis.
- Performance has not been established using cell-free specimens.
- Specimens containing precipitates may give inconsistent results. Such specimens should be rejected prior to testing.
- Do not use heat-treated specimens.
- Specimens with obvious microbial contamination should not be tested.

PROCEDURE

Materials Provided

Kit: **HEMOTEC HCV-1/2 (VNA) EIA** and **HEMOTEC Test**

(plus **REAGENTS** for a complete setup)

The list of accessories required for the **COMBINATION** System is listed in the **COMBINATION** System Manual. A combination of accessories is included with the **COMBINATION** System. If additional accessories are required, they are available in compliance with the **COMBINATION** System; the product may be used with a suitable spectrophotometer, Quantara 8 or Quantara 16.

An optional combination of the following accessories for Quantara 8 and Quantara 16 is required for performance of the tests included:

- Reaction Tray
- Cover Slides
- Aspirate Tubes with Aspirating Controls
- 1 N Substrate Acid, No. 7010 (Anti-HCV 1 and 2) and International Controls

Materials Required but Not Provided

- Procedure requires but similar equipment to deliver 50 µL, 100 µL, 200 µL, 300 µL, 400 µL or 500 µL and 10 µL, 20 µL, 30 µL or 50 µL.
- Device for delivery of reagent solution such as Gilson-Fisher® Dispensing Pump or equivalent.
- Quick-Block™ or device for washing beads such as a PreWashed™ or equivalent to deliver 5 x 15, 30, 45, or 60 wash cycles, such as Gilson-Fisher Pump and/or a wash tray for retaining the cuprate solid containing minimum amount of liquid if necessary.
- The Pre-Quantara™ Wash/Water and Reagent Dispenser may also be used in the performance of this assay.
- COMBINATION Dynamic incubator 200.
- Disposable, graduated pipettes or dispensers for measuring Diluent for OPD.
- Nonmetallic Ruler.
- Quantara 8, Quantara 16, or spectrophotometer capable of reading absorbance at 490 nm with a band width of 10 nm ± 2 nm.
- 30 Pipettes.
- Microtiter Seed Plates for Test for Antibody.

Additional Reagents Available

- Anti-HCV 1-Phenylenediamine + 2 HCl Reagent No. 8110.
- 1 N Substrate Acid, No. 7010.

HEMOTEC HCV-1/2 (VNA) EIA TEST PROCEDURE

Laboratories using the **COMBINATION** System should refer to the appropriate **COMBINATION** Control use Manual and take special **COMBINATION** instructions before.

Precautionary Comments

- Always wear suitable eye and skin + Protective Controls, and face and/or Protective Controls with each set of specimens. Ensure that all reagents kept on hand are subjected to the same process and modification times. This may require modification of specific time and/or incubation time. Do not use the same lot for multiple replicates of subsequent lots without justification.
- HCV 1 Positive Control should be performed in a vial that is fully covered and sealed during the HCV 1 Positive Control. Identify each Positive Control as a tested with a unique value (numbered) used in the laboratory.

Study 2

Specimen	Mean SDC	RRA assay		RFA assay	
		SD	NCV	SD	NCV
1	0.173	0.031	17.8	0.030	30.1
2	0.189	0.056	25.9	0.139	7.3
3	0.201 ^a	0.021	3.7	0.240	7.5
4	0.201 ^a	0.026	3.4	0.476	7.3
5	0.210	0.036	3.6	0.070	6.0

Group	Mean Seroconcordance	RRA assay		RFA assay	
		SD	NCV	SD	NCV
Arginine	0.020	0.000	0.0	0.000	0.0
HIV-1 Positive	1.267	0.261	4.0	0.386	6.8
HIV-1 Negative	0.027	0.003	0.4	0.003	0.4

Sensitivity and Specificity

Study 1 (N = 10,000) was a **prospective, longitudinal, population-based study** of HIV-1 antibody prevalence in the United States.

Sensitivity for HIV-1 antibody assay was calculated based on the clinical diagnosis of AIDS. The HIV-1 antibody assay was compared to a previously licensed test based on comparative studies in various clinical groups including AIDS, ARC, and High Risk.

The specificity studies show that:

1. Specificity based on an assumed zero prevalence of antibody to HIV-1 and/or HIV-2 in specimen donors (17502 out of 10000) is estimated to be 99.99% with a 95% confidence interval: 99.82-99.99% (Table 10).

2. In these calculations, one sample of the specimen total repeatedly reactive specimens was confirmed by Western Blot and has been excluded.

3. HIV-1 seropositivity was equivalent to seropositivity by Western Blot in a population of 102 AIDS patients with known antibodies (302 out of 302 detected for an estimated sensitivity of 100% with a 95% confidence interval: 99.10 - 100%, Table 10). Similarly, the HIV-1 seropositivity was equivalent to a previously licensed test for 1442 seropositive samples from other groups with ARC, High Risk or clinical status unknown (1000 out of 1002 detected for an estimated sensitivity of 100% with a 95% confidence interval: 99.11 - 100%, Table 11).

4. HIV-2 antibody detection rate in a population of 266 HIV-2 confirmed antibody seropositive samples (266 out of 266) is estimated to be 100% with a 95% confidence interval: 99.70 - 100%, compared to a previously licensed HIV-2 EIA which is 99.20% (244 out of 245), Table 10.

B. REACTIVITY IN RANDOM DONOR POPULATION

The reactivity of specimens from specimen blood donors at antibodies to HIV-1 and/or HIV-2 is shown in Table 11. The data include 17054 samples obtained from random blood donors at the geographically diverse blood banks and the seropositivity rates are:

1. HIV-1 antibody reactivity to the **ELISA HIV-1/2 (2.0) EIA** using an indirect and Western Blot (WB) confirmed by the previously licensed HIV-1 EIA. The same 11 samples were reactive by both methods.

2. To determine the ability of the **ELISA HIV-1/2 (1.0) EIA** to detect antibody to HIV-1 and/or HIV-2 in an HIV-2 endemic area, 800 unselected specimens from the Cape Verde Islands were initially tested with both HIV-1 and HIV-2 Western Blots. All specimens were then tested with the **ELISA HIV-1/2 (2.0) EIA**. The results are shown in Table 11.

3. The reactivity of specimens from random blood donors at antibodies to HIV-1 and/or HIV-2 is shown in Table 11. The data include 17054 samples obtained from random blood donors at the geographically diverse blood banks and the seropositivity rates are:

4. HIV-1 antibody reactivity to the **ELISA HIV-1/2 (2.0) EIA** using an indirect and Western Blot (WB) confirmed by the previously licensed HIV-1 EIA. The same 11 samples were reactive by both methods.

5. To determine the ability of the **ELISA HIV-1/2 (1.0) EIA** to detect antibody to HIV-1 and/or HIV-2 in an HIV-2 endemic area, 800 unselected specimens from the Cape Verde Islands were initially tested with both HIV-1 and HIV-2 Western Blots. All specimens were then tested with the **ELISA HIV-1/2 (2.0) EIA**. The results are shown in Table 11.

TABLE 8**Detection of Antibodies to HIV-1 and/or HIV-2 in Serum Specimens and Plasma Specimens from Blood and Plasma Donors**

Number Tested	ELISA HIV-1/2 (2.0) EIA		Western Blot (WB) Confirmed
	Reactive	Positivity Rate (%)	
Number from Random Blood Donors (2 sites)	115 (0.7%)	70 (1.1%)	
Mean (SD) from Volunteer Blood Donors (4 sites)	193 (0.1%)	50 (0.6%)	
Mean (SD) from Plasmapheresis Donors (1 site)	60 (0.1%)	60 (0.1%)	
TOTAL (17054) (2 sites)	270 (0.1%)	160 (1.1%)	

C. REACTIVITY IN PATIENT POPULATIONS

1. **Retrospective Studies**

The reactivity of the **ELISA HIV-1/2 (2.0) EIA** was determined by testing specimens from adults clinically diagnosed as having AIDS, AIDS related complex, opportunistic infections, or were HIV-1 antibody positive patients treated with AZT, and specimens from HIV-2 antibody positive individuals for whom the clinical index was seronegative (Table 9).

TABLE 9**Detection of Antibodies to HIV-1 and/or HIV-2 in Specimens from Individuals Presumptive as Positive for HIV-1 Antibodies**

Group	Number Tested	ELISA HIV-1/2 (2.0) EIA		Previously Licensed	
		Reactive	Positivity Rate (%)		
AIDS	327	352	(108.0%)	248	(80.7%)
ARC	270	270	(100.0%)	270	(100.0%)
Acquired AIDS	192	192	(100.0%)	192	(100.0%)
Primary	69	69	(100.0%)	69	(100.0%)
Medical AIDS AZT	501	501	(100.0%)	501	(100.0%)
Other Donors	1094 ^a	1094	(100.0%)	1086	(99.3%)

^a 245 specimens tested by the licensed HIV-1 EIA due to insufficient volume of serum samples.

^b 1087 specimens tested by the licensed HIV-1 EIA due to insufficient volume of serum samples.

The ability of the **ELISA HIV-1/2 (2.0) EIA** to detect antibodies to HIV-1 and HIV-2 in 245 specimens from a total of 246 seropositive and seronegative individuals, including persons from Europe and West Africa, is shown in Table 10.

TABLE 10**Detection of Antibodies to HIV-1 and/or HIV-2 in Specimens from Individuals Presumptive as Positive for HIV-2 Antibodies and Confirmed by Western Blot**

Group	Number Tested	ELISA HIV-1/2 (2.0) EIA		Previously Licensed	
		Reactive	Positivity Rate (%)	Reactive	Positivity Rate (%)
AIDS	9	9	(100.0%)	9	(100.0%)
ARC	24	24	(100.0%)	24	(100.0%)
Acquired AIDS	83	83	(100.0%)	83	(100.0%)
Other	139 ^a	139	(100.0%)	138	(99.3%)
TOTAL	246 ^b	246	(100.0%)	244	(99.2%)

^a 122 specimens tested by the licensed HIV-2 EIA due to insufficient volume of one sample.

^b 245 specimens tested by the licensed HIV-2 EIA due to insufficient volume of one sample.

2. Prospective Studies

The results of testing for antibody to HIV-1 and/or HIV-2 in 100 specimens from 100 individuals of high risk for HIV-1 infection are shown in Table 10.

TABLE 11**Detection of Antibodies to HIV-1 in Specimens from Individuals of High Risk for HIV Infection in the United States**

Number Tested	ELISA HIV-1/2 (1.0) EIA		HIV-1 EIA	
	Reactive	Positivity Rate (%)	Reactive	Positivity Rate (%)
100	10 ^a	10	10	10

^a 10 (10%) confirmed by the **ELISA HIV-1/2 (2.0) EIA** using an indirect and Western Blot (WB) confirmed by the previously licensed HIV-1 EIA. The same 11 samples were reactive by both methods.

2. To determine the ability of the **ELISA HIV-1/2 (1.0) EIA** to detect antibody to HIV-1 and/or HIV-2 in an HIV-2 endemic area, 800 unselected specimens from the Cape Verde Islands were initially tested with both HIV-1 and HIV-2 Western Blots. All specimens were then tested with the **ELISA HIV-1/2 (1.0) EIA**. The results are shown in Table 11.

3. The reactivity of specimens from random blood donors at antibodies to HIV-1 and/or HIV-2 is shown in Table 11. The data include 17054 samples obtained from random blood donors at the geographically diverse blood banks and the seropositivity rates are:

4. HIV-1 antibody reactivity to the **ELISA HIV-1/2 (2.0) EIA** using an indirect and Western Blot (WB) confirmed by the previously licensed HIV-1 EIA. The same 11 samples were reactive by both methods.

5. To determine the ability of the **ELISA HIV-1/2 (1.0) EIA** to detect antibody to HIV-1 and/or HIV-2 in an HIV-2 endemic area, 800 unselected specimens from the Cape Verde Islands were initially tested with both HIV-1 and HIV-2 Western Blots. All specimens were then tested with the **ELISA HIV-1/2 (2.0) EIA**. The results are shown in Table 11.

TABLE 12**Detection of Antibodies to HIV-2 in Unselected Specimens from an HIV-2 Endemic Area**

Number Tested	ELISA HIV-1/2 (2.0) EIA		HIV-2 EIA	
	Reactive	Positivity Rate (%)	Reactive	Positivity Rate (%)
400	10 (2.5%)	10 (2.5%)	10 (2.5%)	10 (2.5%)

^a 4000 specimens confirmed by Western Blot (WB). 1 specimen was HIV-1 WB positive, 2 specimens were HIV-1 and HIV-2 WB positive, 1 specimen was HIV-2 WB positive. 3999 specimens were reactive to the **ELISA HIV-1/2 (2.0) EIA**. The non-reactive sample was HIV-1 WB positive only.

B. REACTIVITY OF SEROCONVERTING DONORS

Specimens for improved sensitivity of the **ELISA HIV-1/2 (2.0) EIA** were obtained from studies of seroconverting sera (see Table 13). Nine seroconverting pairs were obtained retrospectively from plasmapheresis donors with no known risk factors. All specimens were tested by an HIV-2 licensed Western Blot. All were seropositive by both methods (reactive by both an unlicensed HIV-1 EIA and HIV-2 Western Blot). These donors plus the specimen of 10250, C1708, and H0213 (see Methods described previously.) Each sample was tested in the separate assays except H0213, which was tested in three assays. The **ELISA HIV-1/2 (2.0) EIA** detected the reactivity of HIV-1 antibodies in the same time as other than the WB licensed Abbott HIV-1 HIV-2 EIA.

